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Instability of Hemoglobin Molecule : Unstable Hemoglobins with Substitution at the Heme Contacts—A Review. Part II.

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MOLECULAR BASIS OF UNSTABLE HEMOGLOBINS

The stability and solubility of hemoglobin molecule depends upon an ordered tertiary structure and variety of structural defects can affect the hemoglobin stability. Out of many processes, the most common involves the replacement of those amino acids which are either in direct contact with heme moiety or in the vicinity of heme pocket. Replacement of these hydrophobic residues by polar, charged, hydrophilic residues will result in the lethal distortion of hemoglobin molecule exhibiting either impaired bonding between heme and globin chain, or in some cases the total loss of heme group. Substitution in the interior of folded globin chains e. g. at $\alpha_1\beta_2$ contact would result in weakening of the contacts and increased dissociation of Hb molecule into $\alpha\beta$ subunits which is due to the distortion of the affected subunits. Deletion of certain number of amino acids from the polypeptide chains of hemoglobin would disrupt the secondary structure of the molecule itself. The instability of the molecule is also associated with oxidative changes in the molecule producing methemoglobins. The subunit structure can also be affected due to replacement of a helical residue by a proline residue. This would shift the equilibrium between α helix and random coil in a given segment. Since each subunit of hemoglobin molecule is held by weak non-covalent bonds, loss of some of these linkages can produce the instability of Hb molecule. The instability of Hb molecule sometime may be self-caused e.g. the substituted amino acid may have an extraordinary large side chain which is difficult to be accommodated in the interior of the subunit. Alternatively, the incoming amino acid may have a smaller side chain which fails to make contact with neighbouring amino acids. All the above mentioned mechanisms responsible for the instability of hemoglobin molecule will be discussed in detail in the following sections but it must be remembered that there are certain exceptions in which the relationship between the instability and structural changes is not apparent and difficult to explain.

AMINO ACID SUBSTITUTION IN THE HEME POCKET

There are three clusters of hydrophobic residues near the heme, one on

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the side of E7 (distal histidine) residue ; the other one on the side of F8 (proximal histidine) residue and the third one at the bottom of heme. These clusters of hydrophobic amino acids are located in CDEF and FG helices, the central region of the molecule. These helices have their own significance because porphyrin interacts with a number of non polar hydrophobic amino acids in these regions of subunits. Interestingly, of the total 109 presently known unstable variants, 50 involve the substitution in the central region of the Hb molecule. The principal forces responsible for the configuration and stability of hemoglobin are the hydrophobic bonds formed by internally located invariably non-polar residues. The replacement of these internal hydrophobic residues by other non polar residues of different dimensions can produce a different Hb molecule having variable degree of instability not totally compatible with its survival. The loss of important hydrophobic heme-globin contact loses the hold of the globin on heme. This conformational change would permit water to enter into heme pocket and in the presence of oxygen and water, hemoglobin is oxidised to methemoglobin. The oxidation of unstable hemoglobin to methemoglobin is followed by a subsequent oxidation of the β93 cysteine. Blockading of titrable -SH groups results in distortion of the molecule giving rise to oxidation of other SH groups finally causing the precipitation of the molecule. The continuous formation of oxidative products may explain the decreased levels of GSH found in the red cells of patients with unstable hemoglobins.

There are 32 unstable hemoglobins in which heme contact is affected and among these included 27 variants in which the amino acid residues of central region (CDEF and FG helices) which are also heme linked have been substituted (Table 1). Most of these mutants involve the replacement of one internal hydrophobic residue by another, because a fully charged group in this critical central region would lead to a totally non-viable hemoglobin molecule. For example when phenylalanine (Phe) (CD1 or CE1) which makes contact with heme is replaced by another hydrophobic residue such as valine as in Hb Torino [α43(CE1) Phe→Val][7] or by leucine as in Hb Hirosaki [α43(CE1) Phe→Leu][8] or Hb Louisville [β42(CD1) Phe→Leu][9–12] mild to moderate hemolytic process is reported in the carriers which is compensated after splenectomy. But when same phenylalanine is replaced by polar, charged hydrophilic residue serine as in Hb Hammersmith [α42(CD1) Phe→Ser][13–16] a severe uncompensated hemolytic anemia in the propositus has been described.

The substitution in Hb Fort de France [α45(CE3) His→Arg][17] involves the replacement of an identical positively charged histidine by arginine. Individual with Hb Fort de France abnormality presents a mild instability of the Hb molecule. A mild Heinz body hemolytic anemia and splenomegaly was reported in a Turkish who was a carrier for Hb Moabit [α86(F7) Leu→Arg].[18] The leucine α86 (F7) which is an non-polar residue is in heme contact and it has been replaced by polar amino acid in the interior of heme pocket weakening the heme binding and allowing the water to enter into the hydrophobic heme pocket. F7 (α86) is also next to the proximal histidine (F8) and located at the helical part of the polypeptide chain molecule and this replacement would markedly affect the stability of the hemoglobin molecule. A substitution of F7 has also been reported in Hb Sabine [β91(F7) Leu→Pro][19], a β-chain variant

where a different mechanism is responsible for severe hemolytic disorder as compared to mild and nearly compensated hemolytic anemia in Turkish patient with Hb Moabit.[18]

A severe transient drug induced hemolytic crisis was observed in a carrier of Hb Mequon [β41(C7) Phe→Tyr][20] who was treated with acetoaminophen for viral illness. The anemia as we see is drug-induced because the substitution of Phe→Tyr should not affect the stability of the molecule because both of the amino acid residue (Phe and Tyr) have similar volume and hydrophobicity. Hb Zürich [β63 (E7) His→Arg][21–25] is moderately unstable and shows some accelerated auto-oxidation. The substantial tolerance of the red cells to Hb Zürich has been explained by a possible pushing out of the charged arginine residue to the surface of the molecule. From the hematological data it is moderately regenerative and in fact the anemia becomes evident on drug induction. Hb Toulouse [β66(B10) Lys→Glu][26–28] involves the substitution of an amino acid linked to a propionic group of heme rupturing an ionic bond in the heme pocket. The β67(E11) is a valine residue in Hb A which lies on the side of E helix facing the non-polar pocket in the globin chain which contains the heme group, and both γ carbon atoms of this residue make contacts with the heme group. Two mutations have been reported at this position E11, the hydrophobic interior of the molecule. They are, Hb Bristol [β67(E11) Val→Asp][29] and Hb Sydney [β67(E11) Val→Ala].[25,30] The replacement of one non-polar residue by a charged aspartyl residue as in Hb Bristol in the hydrophobic interior of the molecule is energetically unfavourable and is likely to produce considerable rearrangement of this part of molecule. In Hb Sydney, alanine produces some instability of the hemoglobin molecule, this is largely due to the loss of a polar bonds between the valyl residue and the heme group. The clinical picture in Hb Sydney and Hb Bristol is that of chronic hemolytic anemia, more intense in Hb Bristol not compensated even after splenectomy than in Hb Sydney disease. This suggests that Hb Bristol is a less stable hemoglobin than Hb Sydney which correlates well with the known amino acid substitution in these two hemoglobinopathies.

The structural abnormality in Hb Seattle [β70(E14) Ala→Asp][31,32] is due to substitution of aspartic acid for alanine which is in heme contact but located at the surface of the molecule. This explains both the mild instability and mild compensated hemolytic anemia observed in the propositus. The phenylalanine β71(E15) forms a Van der Waals contact with heme ; its replacement by more polar serine as in Hb Christchurch [β71(E15) Phe→Ser][33] would result in greater release of heme moiety and access of water to heme pocket. The net effect will be release of free globin, subsequent precipitation of the red cell and oxidation to Heinz bodies formation. In Hemoglobin Böras [β88(F4) Leu→Arg][34,35] leucine has been replaced by arginine which has sufficiently long and flexible side chain for the charged group to be carried to the exterior of the molecule. The guanidinium group in Hb Böras can be accommodated in a crevice at the outside of the molecule while the δ carbon of the arginine can make at least one of the two δ carbon hydrophobic contacts which are normally provided by leucine. Hb Caribbean [β91(F7) Leu→Arg][36] is mildly unstable hemoglobin. The leucine β91 (F7) is next to the proximal histidine (F8) and located at the surface crevice but its side chain is directed towards the heme.

The side chain of the incoming arginine is most probably accommodated at this position by orientation of the side chain so that the guanidinium group is placed at the surface of the molecule. One can also speculate that most probably the heme contact made by F7 leucine is not fully lost in this variant and it might still be possible for guanidinium group to interact with ε-NH_2 group of lysine β66 (E10).

The replacement of the proximal histidine (F8) must have serious effects on the binding of heme group with its globin chains. The proximal histidine (F8) is a key amino acid residue in forming a unique spatial structure in the hemoglobin subunits by binding ferrous atom of the heme and also participating in α_1-β_2 contact. Any substitution at this point is bound to affect the structural and functional properties of Hb molecule. For example in Hb Istanbul [β92(F8) His→Gln][37] the hemoglobin is not capable of binding heme to the abnormal globin chains and behaves as seminatural hemoglobin. It will be interesting to note that the replacement of histidine by aspartic acid in Hb Altgeld Gardens [β92(F8) His→Asp][38] produces a mild functional disturbance. The propositus of Hb Altgeld Gardens presents a long life anemia otherwise asymptomatic. Hb Mozhaisk [β92(F8) His→Arg][39] was reported in Russian heterozygote in which positively charged histidine has been replaced by similar charged arginine. This unstable Hb exhibits altered functional properties and has only two heme groups per tetramer. A severe anemia, jaundice and marked hepatosplenomegaly has been reported in the carrier.

The valine β98(FG5) is one of the few invariant residues and is particularly important because it not only forms direct contact with the heme but also involved in α_1-β_2 contact. Hb Köln [β98 (FG5) Val→Met][40–52] is most frequently reported unstable hemoglobin all over the world and in many ethnic groups. The structural studies on Hb Köln has shown the replacement of smaller valine (β98) by methionine with larger side chain. This molecular perturbation results in heme loss and increased oxygen affinity. The severe hemolytic anemia with marked marrow expansion and bizzare blood film was observed in a patient with Hb Nottingham [β98 (FG5) Val→Gly].[53] The substitution of glycine alters the heme contact because of absence of the side chain. In Hb Nottingham glycine fails to make the contact with heme, therefore precipitates more rapidly than Hb Köln. Hb Djelfa [β98 (FG5) Val→Ala][54,55] involves the replacement of a hydrophobic amino acid by another and regarded as mildly unstable with no clinical consequence. The carriers of Hb Tübingen [β106(G8) Leu→Gln][56,57] suffer from a mild compensatory hemolytic anemia with mild cyanosis. The structural abnormality in Hb Tübingen is replacement of leucine by a polar, hydrophilic glutamine which will affect the tertiary structure of molecule. The glutamine is a helical forming amino acid and does not disturb the conformation but possibly will impair the heme contact at position G8(β106). Hb Olmsted [β141(H19) Leu→Arg][58,59] was reported by Fairbanks et al. in 1969. The severe hemolysis observed in the propositus can be explained on the basis that a non-polar residue has been replaced by a charged residue and that would certainly result in a totally non viable hemoglobin molecule.

Apart from the above mentioned unstable hemoglobins, there are some other unstable variants in which one or more heme contact amino acids has

been affected. They are Hbs Niteroi,[60] Gun Hill[61–63] and Coventry[64] which have the deletion of one or more amino acid residues. In Hbs Biba,[65] Yokohama,[66] Bicêtre,[67] Santa Ana,[68,69] Sabine,[19] New Castle,[70] Southampton[71] and Casper[72] a proline residue has been substituted. Hb M Saskatoon[73–75] and Hb M-Hyde Park[76–78] both are methemoglobins of β chain anomaly in which histidine (distal or proximal) has been replaced by tyrosine. The mechanism for their instability and related clinical effects will be discussed in the appropriate sections of this text. (To be continued)

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Table 1 Unstable Hemoglobins with substitutions at the heme contacts

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Functional properties: Bohr effect	Clinical symptoms remarks	References
1. Torino α43(CE1)Phe→Val	heme	E	2.9-3.5	9.0-11.2	27-33	92-93		30-33	4.5-12.2	12	+	+	+	+	1.3	20	Italian	Like A		↓			Moderate to severe hemolytic anemia compensated after splenectomy.	7
2. Hirosaki α43(CE1)Phe→Leu	heme	E	2.6-2.7	7.9-8.2	29-31				11.9-17.6		+	+		+	2.7-3.1	3-11	Japanese	Like A					Congenital non-spherocytic hemolytic disease.	8
3. Fort de France α45(CE3)His→Arg	heme	E	5.1	12.2	44						+			0		20	French W. Indian	Like S		↑	Normal	Normal	Slightly increased precipitation. No. abnormal hematological features.	17
4. *Moabit α86(F7)Leu→Arg	heme	E	4.2-4.8	12.4-14.0			28.6-30.1		6-10	14	+	+		+		15	Turkish	Between S and F		↓			Mild compensated hemolytic syndrome.	18
5. *Biba α136(H19)Leu→Pro	heme	I	2.6-2.7	6.5-7.5	26-28				5.8-16		+	+		+		11	Caucasian	Like S					Hereditary non-spherocytic anemia. Not compensated after splenectomy.	65
6. Yokohama β31(B13)Leu→Pro	heme	I	2.4	7.9	27				50-60	3	+	+		+			Japanese	Like A					Chronic partially compensated hemolytic anemia.	66
7. Mequon β41(C7)Phe→Tyr	heme	SC		6.6	21	120	39	31	22-38.5	12.5	+	0	+	+		40-50	English	Like A		Normal			Severe hemolytic crisis; persistent reticulocytosis.	20
8. Hammersmith (Chiba) β42(CD1)Phe→Ser	heme	SC		6.2					46	2	+	+		+			English Japanese	Like A	1.10	↓			Severe hemolytic anemia not compensated after splenectomy. Cyanosis.	13-16
9. Louisville (Bucuresti) β42(CD1)Phe→Leu	heme	SC	3.8-4.4	11.5-13.5	34-41	89-99	29.3-33.3	32.2-33.1	16.8-9.5	9	+	+		+	↑	35-50	American Rumanian Cuban Canadian	Like A		↓	↓	Normal	Mild hemolysis compensated after splenectomy.	9-12
10. M. Saskatoon β63(E7)His→Tyr (Distal His)	heme	SC									+									Normal	↓	Present	Cyanosis, anemia.	73-75
11. Zürich β63(E7)His→Arg (Distal His)	heme	SC		11-14.7	34-38				2.8	11-13	+	Int.	+	+		25	Swiss	Like S		↑	↓	Normal	Mild hemolytic disease. Severe hemolysis after sulfonamide therapy.	21-25
12. Bicêtre β63(E7)His→Pro (Distal His)	heme	SC	2.6	10.7		127		32.5	2.3		+	+	+		2.0		French	Like A		Normal	↓		Highly regenerative hemolytic anemia.	67
13. *Toulouse β66(E10)Lys→Glu	heme	E	3.3	15.7	42-46	120			1.4	13	+	+				40	French	Faster than A		Normal	Normal	Normal	Unstable	26-28
14. *Bristol (Niigata) β67(E11)Val→Asp	heme	I	2.5	8.0	31	124	32	26	80		+	+	+			36	English	Like A		↓	↓	↓	Permanent severe hemolytic anemia not compensated after splenectomy.	29
15. Sydney β67(E11)Val→Ala	heme	I									+		+				German	Like S					Chronic hemolytic diethesis. (Mild hemolytic disease except during crisis.)	30
16. Seattle β70(E14)Ala→Asp	heme	E		8.7-11.6	27-33				2.0	16	+		+			39-43	Caucasian	Like J		↓	Normal	Normal	Mild compensated hemolytic anemia.	31,32
17. Christchurch β71(E15)Phe→Ser	heme	I			5.5-10.5				8-15		+		+			22	Australian	Like A					Moderate to severe hemolytic anemia compensated after splenectomy.	33
18. Böras β88(F4)Leu→Arg	heme	SC	3.6	12.6		120		28	3.5		+	+	+		2.2	10	Swedish	slightly slower than A			↓		Moderate to severe hemolysis compensated after splenectomy. Increased methemoglobin formation.	34,35

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Functional properties: Bohr effect	Clinical symptoms remarks	References
19. *Santa Ana β88(F4)Leu→Pro	heme	SC	3.5	9.0					16-21		+	+	+	+		5	American Hungarian	Like S					Hereditary non-spherocytic hemolytic anemia well compensated after splenectomy.	68,69
20. *Sabine β91(F7)Leu→Pro	heme	SC	2.4-2.9	8.5-10.5	28-36				35-67	4.0	+	+	0	+	2.4	11	English German	Between S and C					Severe hemolytic anemia not compensated after splenectomy. Rapidly precipitated within red cells.	19
21. Caribbean β91(F7)Leu→Arg	heme	SC	3.3-6.7	9.7-11.7	29-36	86-93	28-29	30-33			+					39	W. Indian	slightly slower than S to Anode		↓		almost absent	Midly unstable. No clinical symptoms.	36
22. M-Hyde Park (M-Akita) β92(F8)His→Tyr (Prox. His)	heme		4.5	12.5	39.5	87	27.6	31.6	5.8	11.5	+	0		+		36	American Norwegian Yugoslavian Japanese	slightly faster than A_2 with a major band anode to Hb A		↑	↓		Cyanosis anemia.	76-78
23. *Istanbul (Saint Entienne) β92(F8)His→Gln (Prox. His)	heme		3.6	9.1	32	88	25	28	4.2		+	+				12-15	Turkish French	Between A_2 and S		↑	no heme on α-chain	Absent	Moderate to severe hemolysis compensated after splenectomy.	37
24. *Altgeld Gardens β92(F8)His→Asp (Prox. His)	heme			9.5							+						Black American	Like J		Normal	↓		Life long hemolytic anemia otherwise asymptomatic.	38
25. New Castle β92(F8)His→Pro (Prox. His)	heme			7-7.6					18		+	+		+		17	English	Between A_2 and S					Chronic anemia recurrent jaundice slightly improved after splenectomy.	70
26. *Mozhaisk β92(F8)His→Arg (Prox. His)	heme										+	+				17	Russian	slower than A_2		↑	Normal		Severe anemia; Jaundice and marked hepatosplenomegaly.	39
27. *Köln (Ube-1) β98(FG5)Val→Met	heme (side chain) $\alpha_1\beta_2$ (main chain)	I		12.3-14.6					7-16	7.3	+	+	+	+		10-20	German Japanese and many others	slightly slower than S	1.2-1.4	↑	↓		Moderate hemolytic anemia compensated after splenectomy.	40-52
28. *Nottingham β98(FG5)Val→Gly	heme (side chain) $\alpha_1\beta_2$ (main chain)	I	2.0	6-7	24				49		+	+		+			English	Between S and A		↑	↓		Severe hemolysis.	53
29. *Djelfa β98(FG5)Val→Ala	heme (side chain) $\alpha_1\beta_2$ (main chain)	I									+					30	French	Anodal to A_2 (deheminised)		↑	↓	Normal	Unstable.	54,55
30. Southamptom (Casper) β106(G8)Leu→Pro	heme	I	1.7-3.2	4.5-11.0	19-35				42-94	2.2	+	+	+	+	9.3	40	English American	Like A		↑			Acute hemolytic anemia with increased reticulocytosis.	71,72
31. *Tubingen β106(G8)Leu→Gln	heme	I	4.7	15.5	45	95	32.8	34.5	16-40	14	+		0		0.2-0.8	40	German	Between S and F		↑	↓	Normal	Variable compensated hemolysis. Mild cyanosis.	56,57
32. *Olmsted β141(H19)Leu→Arg	heme	I		4.8					7		+	+				5-10	English	Like S					Severe hemolysis not compensated after splenectomy.	58,59